

Supplementary Information for

The Cytoplasmic Heme Binding Protein PhuS of *P. aeruginosa* is a Heme Oxygenase Titratable Regulator of Heme Uptake.

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Table S1. Bacterial strains and plasmids.

Strain	Description	Source or reference
<i>E. coli</i> BL21(DE3)	F ⁻ dcm ompT hsdS(r _B ⁻ m _B ⁻) galλ (DE3)	Stratagene
<i>P. aeruginosa</i> PAO1	Wild type	¹ Holloway BW, 1955
PAO1 <i>ΔhemO</i>	PAO1 <i>hemO::aacC1</i> Gm	² Oglesby-Sherrouse & Vasil, 2010
PAO1 <i>ΔphuS</i>	PAO1 <i>ΔphuS</i>	³ Barker et al, 2012
Plasmids MRL2	Amp ^R ;pET-11a derivate harboring the rat liver outer mitochondrial membrane cytochrome <i>b₅</i> gene encoding a water-soluble domain of the cytochrome b ₅	⁴ Rivera et al., 1995

1. Holloway, B. W. *J Gen Microbiol* **1955**, 13, 572-581.
2. Oglesby-Sherrouse, A. G.; Vasil, M. L. *PLoS One* **2010**, 5, e9930.
3. Barker, K. D., Barkovits, K., Wilks, A. *J Biol Chem* **2012**, 287, 18342-18350.
4. Rivera, M.; Walker, F. A. *Anal Biochem* **1995**, 230, 295-302.

Table S2. Primers and probes used in this study.

<u>Primers and Probes</u>	<u>Sequence</u>
<i>bphO</i> probe	5'-/56-FAM/CCC GGC AGA TCG ACA GCC CC/3BHQ_1/-3'
<i>bphO</i> F primer	5'-GCG CTG GCA GGA GTT TCT C-3'
<i>bphO</i> R primer	5'-ATC GAC GAA ACG AAA GGA ATG T-3'
<i>phuS</i> probe	5'-/56-FAM/CTT TCG GCC GCC GCT TCG A/3BQH_1/-3'
<i>phuS</i> F primer	5'-TGC CGA CGA ACA CCA TGA-3'
<i>phuS</i> R primer	5'-TGG CGA CCT GGC GAA A-3'
<i>hemO</i> probe	5'-/56-FAM/TTC GTC GCC/ZEN/GCC CAG TAC CTC TTC CAG CAT/3IAB1kFQ/3'
<i>hemO</i> F primer	5'-TGG TGA AGA GCA AGG AAC CCT TC-3'
<i>hemO</i> R primer	5'-TTC GTT GCG ATA AAG CGG CTC CA-3'
<i>phuR</i> probe	5'/56-FAM/TAC GCG CAG ACC CAC CGC AA/3BQH_1/-3'
<i>phuR</i> F primer	5'-ACT GCC CAA CGA CTT CTT CAG-3'
<i>phuR</i> R primer	5'-TTA CGA TGT CCG GAT CGA CGT A-3'

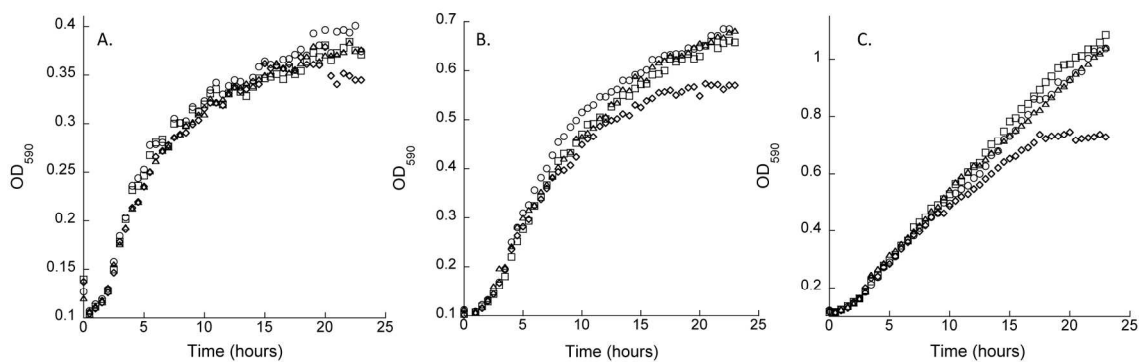


Figure S1. Growth curves of wild type and mutant PAO1 strains in the presence of heme. Cultures were grown in M9 minimal media supplemented with (A) 0 μ M heme, (B) 0.5 μ M heme (C) 5.0 μ M heme. Growth curves for PAO1 (○), $\Delta phuS$ (□), $hemO$ (◇), and $\Delta phuS/hemO$ (Δ).

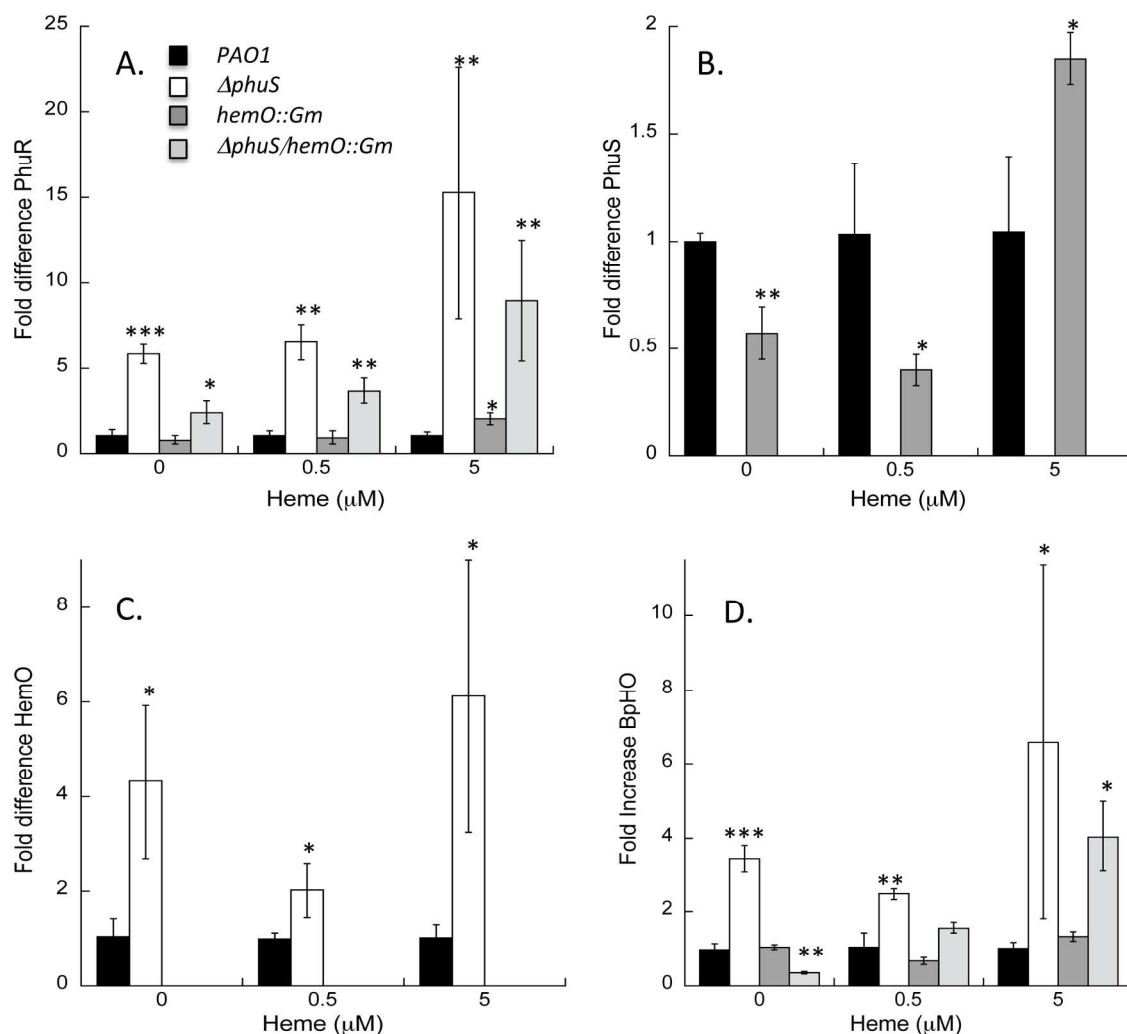


Figure S2. Relative expression of the heme utilization proteins in the PAO1 $\Delta phuS$, $\Delta hemO$ and $\Delta phuS/\Delta hem$ strains compared to wild type. (A) PhuR, (B) PhuS, (C) HemO, and (D) BphO. RNA isolated from the indicated strains following 8 hours growth in media supplemented with heme as indicated was analyzed as described in the Methods. The data represents the standard deviation from at least three independent experiments in triplicate. p -values for the mRNA levels of the individual genes in the deletion strains were normalized to the levels in PAO1 where * $p < 0.05$, ** $p < 0.005$ or *** $p < 0.001$.

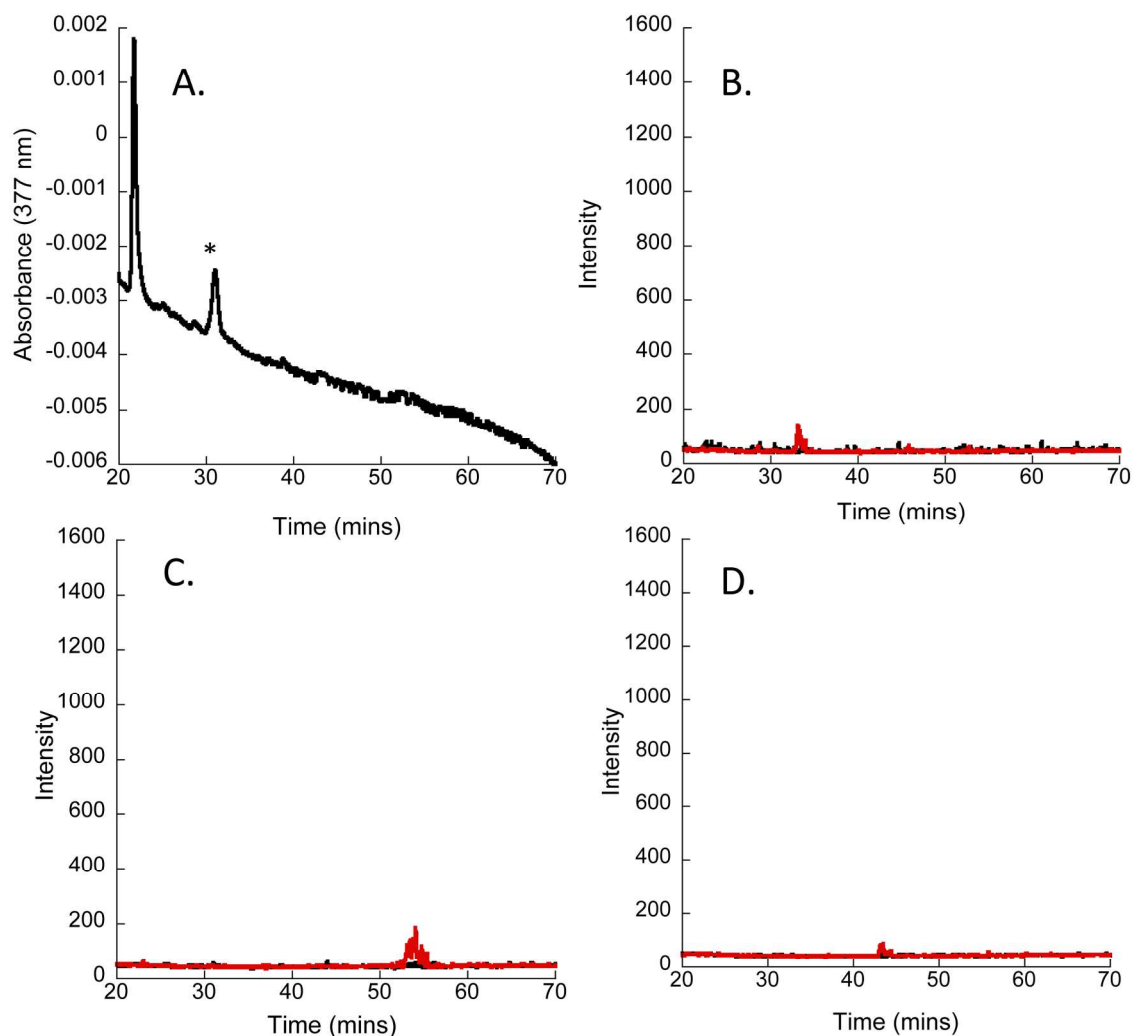


Figure S3. LC-MS/MS BVIX isomer fragmentation patterns for PAO1 *AphuS* supplemented with $0.5\ \mu\text{M}$ ^{13}C -heme. (A) HPLC analysis of BV isomers following extraction from the extracellular media. BVIX isomer peaks as marked. *Indicates a non-BVIX contaminant; (B) MS/MS fragmentation of ^{13}C -BVIX α (red line) and ^{12}C -BVIX α (black); (C) MS/MS fragmentation of ^{13}C -BVIX δ (red line) and ^{12}C -BVIX δ (black); MS/MS fragmentation of ^{13}C -BVIX β (red line) and ^{12}C -BVIX β (black). LC-MS/MS was performed as described in the Methods with multiple reaction monitoring.

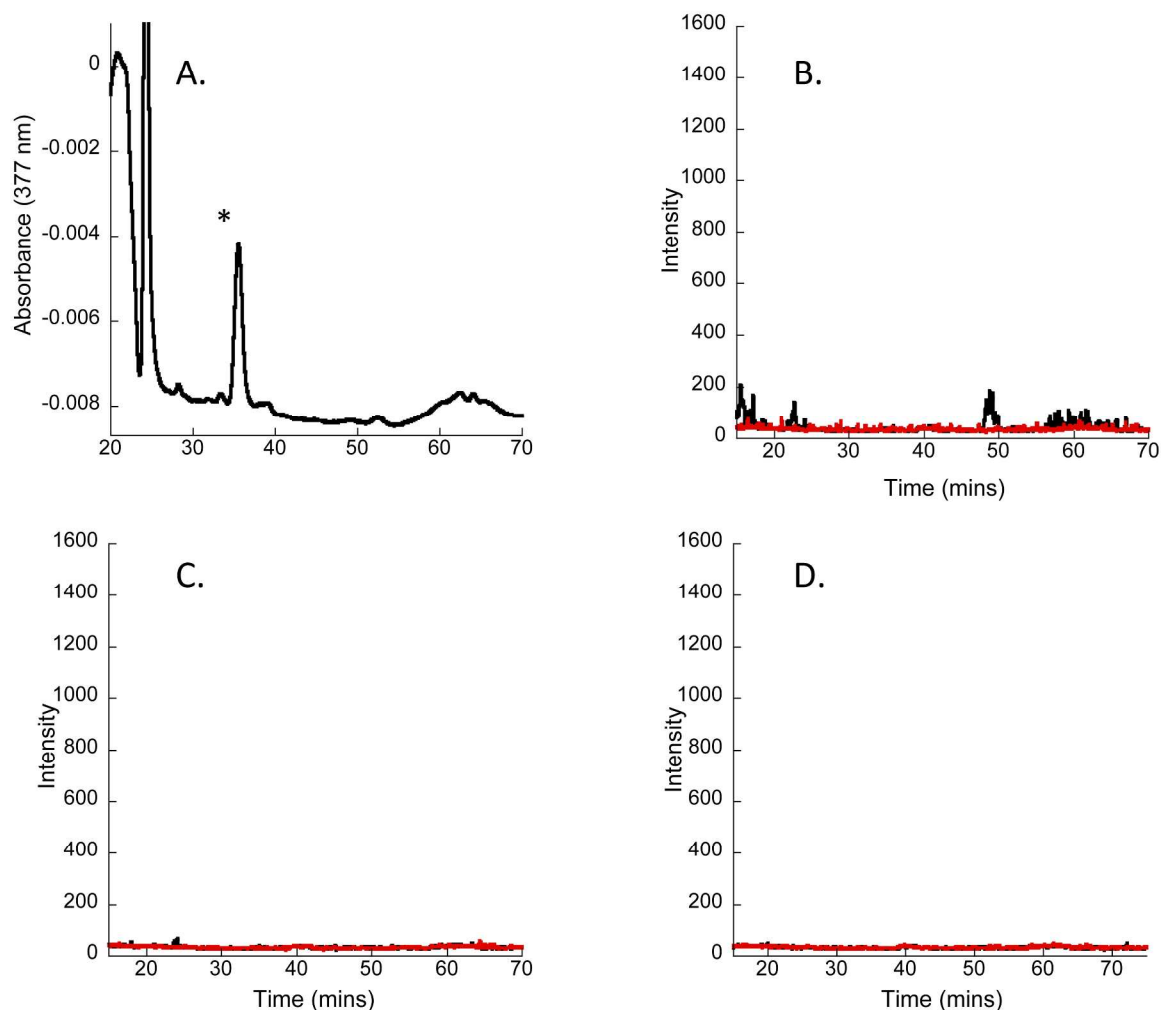


Figure S4. LC-MS/MS BVIX isomer fragmentation patterns for PAO1 *ΔhemO* supplemented with 0.5 μM ^{13}C -heme. (A) HPLC analysis of BV isomers following extraction from the extracellular media. BVIX isomer peaks as marked. *Indicates a non-BVIX contaminant; (B) MS/MS fragmentation of ^{13}C -BVIX α (red line) and ^{12}C -BVIX α (black); (C) MS/MS fragmentation of ^{13}C -BVIX δ (red line) and ^{12}C -BVIX δ (black); MS/MS fragmentation of ^{13}C -BVIX β (red line) and ^{12}C -BVIX β (black). LC-MS/MS was performed as described in the Methods with multiple reaction monitoring.

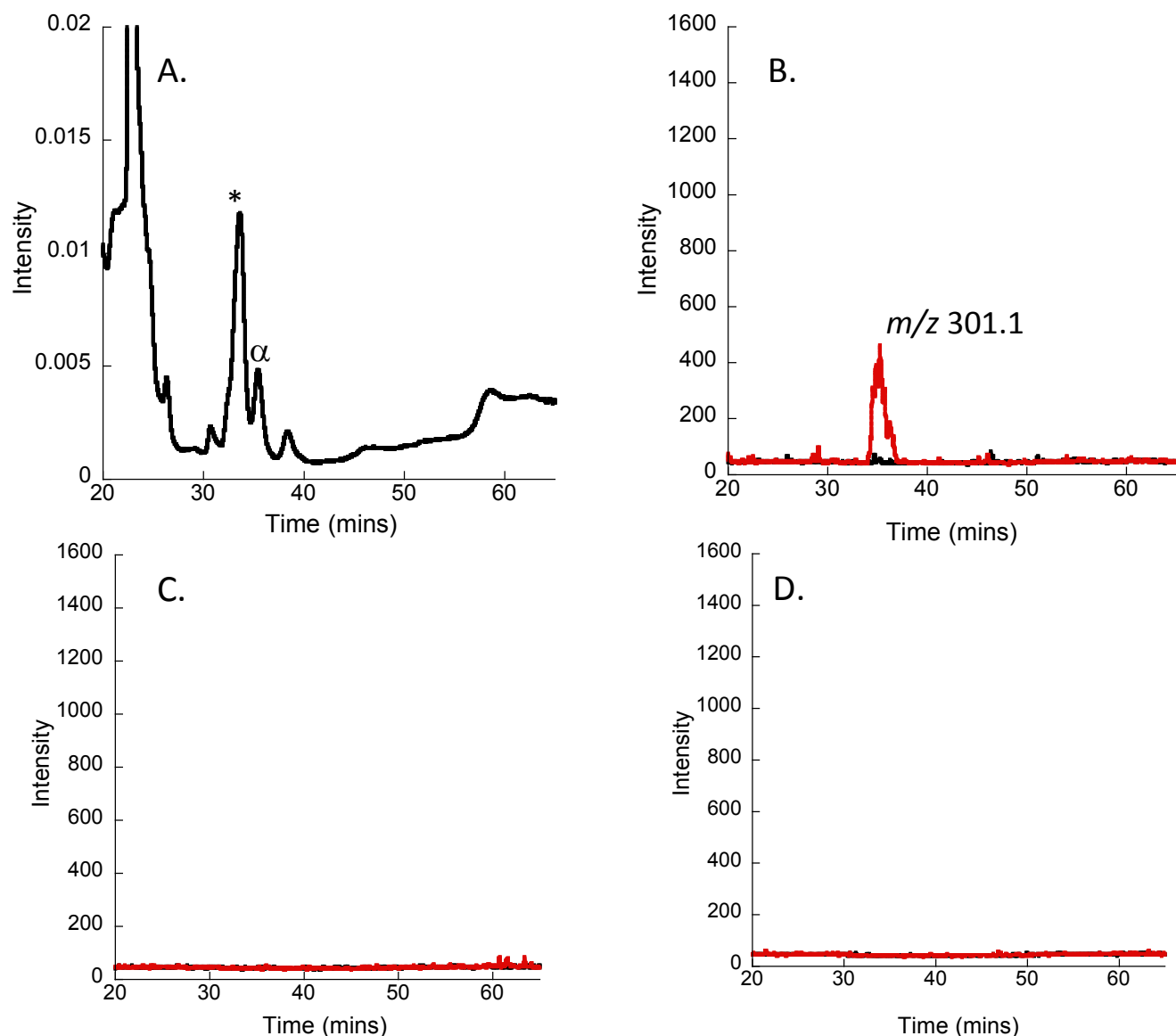


Figure S5. LC-MS/MS BVIX isomer fragmentation patterns for PAO1 *AphuS*/*AhemO* supplemented with 0.5 μM ^{13}C -heme. (A) HPLC analysis of BV isomers following extraction from the extracellular media. BVIX isomer peaks as marked. *Indicates a non-BVIX contaminant; (B) MS/MS fragmentation of ^{13}C -BVIX α (red line) and ^{12}C -BVIX α (black); (C) MS/MS fragmentation of ^{13}C -BVIX δ (red line) and ^{12}C -BVIX δ (black); MS/MS fragmentation of ^{13}C -BVIX β (red line) and ^{12}C -BVIX β (black). LC-MS/MS was performed as described in the Methods with multiple reaction monitoring.